Direct interaction between HLA-B and carbamazepine activates T cells in patients with Stevens-Johnson syndrome

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Background: Increasing studies have revealed that HLA alleles are the major genetic determinants of drug hypersensitivity; however, the underlying molecular mechanism remains unclear.
Objective: We adopted the HLA-B*1502 genetic predisposition to carbamazepine (CBZ)–induced Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) as a model to study the pathologic role of HLA in delayed-type drug hypersensitivity.
Methods: We *in vitro* expanded CBZ-specific cytotoxic T lymphocytes (CTLs) from patients with CBZ-induced SJS/TEN and analyzed the interaction between HLA-B and CBZ analogs based on CTL response, surface plasmon resonance, peptide-binding assay, site-directed mutagenesis, and computer modeling.
Results: The endogenous peptide–loaded HLA-B*1502 molecule presented CBZ to CTLs without the involvement of intracellular drug metabolism or antigen processing. The HLA-B*1502/peptide/β_2-microglobulin protein complex showed binding affinity toward chemicals sharing 5-carboxamide on the tricyclic ring, as with CBZ. However, modifications of the ring structure of CBZ altered HLA-B*1502 binding and CTL response. In addition to HLA-B*1502, other HLA-B75 family members could also present CBZ to activate CTLs, whereas members of the HLA-B62 and HLA-B72 families could not. Three residues (Asn63, Ile95, and Leu156) in the peptide-binding groove of HLA-B*1502 were involved in CBZ presentation and CTL activation. In particular, Asn63 shared by members of the B75 family was the key residue. Computer simulations revealed a preferred molecular conformation of the 5-carboxamide group of CBZ and the side chain of Arg62 on the B pocket of HLA-B*1502.
Conclusions: This study demonstrates a direct interaction of HLA with drugs, provides a detailed molecular mechanism of HLA-associated drug hypersensitivity, and has clinical correlations for CBZ-related drug–induced SJS/TEN. (J Allergy Clin Immunol 2012;129:1562-9.)

Key words: *Carbamazepine, drug hypersensitivity, HLA, Stevens-Johnson syndrome, toxic epidermal necrolysis*

T cell–mediated delayed-type drug hypersensitivity appears as diverse clinical manifestations, including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). SJS and TEN are life-threatening drug hypersensitivities that are characterized by serious blistering reactions in the skin and mucous membranes with systemic complications and multiple organ involvement.[1] SJS/TEN lesions are induced by the migration of circulating skin-homing cytotoxic T lymphocytes (CTLs) that are activated, proliferate, and release cytotoxic proteins to induce keratinocytes apoptosis.[2,3]

Recently, increasing HLA alleles are reported to associate with different kinds of drug hypersensitivity. For example, HLA-B*1502 is strongly associated with carbamazepine (CBZ)–induced SJS/TEN,[4] HLA-B*5801 with allopurinol-induced SJS/TEN/hypersensitivity syndrome (HSS),[5] HLA-B*5701 with abacavir hypersensitivity[6] or flucloxacillin-induced liver injury,[7] and HLA-DRB1*1501 with lumiracoxib-induced hepatotoxicity.[8] Nevertheless, in spite of the strong genetic associations, how a specific HLA allele plays its pathologic role in the initiation of the immune reaction of drug hypersensitivity is not yet well characterized.

Several hypotheses have been proposed to explain the interaction of HLA, drugs, peptides, and T-cell receptors (TCRs). The hapten/prohapten hypotheses propose that a chemically active drug/metabolite forms a covalent bond with an endogenous peptide and then is intracellularly processed and presented by the particular HLA as seen in the classical peptide antigen pathway.[9] For example, HLA-B*5701 was reported to present abacavir to T cells through a tapasin- and processing-dependent pathway.[10] By comparison, the p-i concept (direct pharmacologic interaction of a drug with immune receptors) implies that the direct and reversible interaction of drugs with TCRs or peptide-loaded HLA initiates immune reactions.[11] However, there are very few examples proving the hypotheses.

The genetic predisposition of the HLA-B*1502 allele to CBZ-induced SJS/TEN in Han Chinese patients is the strongest HLA-disease association reported thus far ($P = 1.6 \times 10^{-41}$; odds ratio, 1357; 95% CI, 193.4-8838.3).[4,12] The same association has also

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Abbreviations used
AED: Antiepileptic drug
APC: Antigen-presenting cell
B-LCL: EBV-transformed B-cell line
CBZ: Carbamazepine
CTL: Cytotoxic T lymphocyte
ECBZ: Carbamazepine 10,11-epoxide
ESL: Eslicarbazepine acetate
5HB: 5H-dibenzazepine
HSS: Hypersensitivity syndrome
LIC: Licarbazepine
LTT: Lymphocyte transformation test
MCSS: Multiple copy simultaneous search
OXC: Oxcarbazepine
SJS: Stevens-Johnson syndrome
SPR: Surface plasmon resonance
TCL: T-cell line
TCR: T-cell receptor
TEN: Toxic epidermal necrolysis

been replicated in other populations, particularly in patients from Southeast Asian countries, where the HLA-B*1502 allele is frequent.[13-15] Recently, we reported that a prospective genetic screening of HLA-B*1502 before CBZ treatment could reduce the incidence of CBZ-induced SJS/TEN.[16] It is interesting to note that CBZ-induced SJS/TEN has also been reported to associate with the different members of the HLA-B75 family in populations in regions where HLA-B*1502 is rare.[15,17-19] The HLA association is phenotype specific because CBZ-induced maculopapular eruption/HSS has been reported to associate with HLA-A*3101 in different populations.[12,20,21] Because HLA alleles are the major genetic determinants of drug hypersensitivity, here we adopted the model of the association between HLA-B*1502 and CBZ-induced SJS/TEN to study the pharmacoimmunologic mechanism of HLA in patients with drug hypersensitivity.

METHODS

Clinical samples

We enrolled 20 patients with CBZ-induced SJS/TEN (19 patients with CBZ-induced SJS and 1 patient with CBZ-overlapping SJS and TEN, all of whom were HLA-B*1502 positive) and 4 CBZ-tolerant control subjects who were HLA-B*1502 positive and had been administered CBZ for at least 3 months without any cutaneous adverse reaction from Chang Gung Memorial Hospital and National Taiwan University Hospital, Taiwan (see Table E1 in this article's Online Repository at www.jacionline.org). All patients with SJS/TEN were accessed through review of photographs, pathologic slides, and medical records by 2 dermatologists in this study. Approval was obtained from the institutional review board, and informed consent was obtained from each participant.

Chemicals, cell lines, and CBZ-specific T cells

We purchased CBZ, carbamazepine 10,11-epoxide (ECBZ; the reactive metabolite of CBZ), oxcarbazepine (OXC; a second-generation drug to CBZ), eslicarbazepine acetate (ESL; a third generation drug to CBZ), licarbazepine (LIC; the active metabolite of OXC or ESL), 5H-dibenzazepine (5HB; also known as iminostilbene), and gabapentin (a nonaromatic antiepileptic drug [AED] used as the control drug) from Sigma-Aldrich (St Louis, Mo). These chemicals are illustrated in Fig E1 in this article's Online Repository at www.jacionline.org. We used EBV-transformed B-cell lines (B-LCLs), an HLA class I–deficient lymphoblastoid cell line (C1R), and a keratinocyte cell line (KERTr) as antigen-presenting cells (APCs). We overexpressed different HLA-B proteins in C1R or KERTr cells by transfecting full-length or soluble cDNAs using Effectene (Qiagen, Hilden, Germany) or electrophoresis. CBZ-specific T-cell lines (TCLs) were obtained by culturing the patients' PBMCs with CBZ (25 μg/mL) for 10 to 14 days, and the expanded T cells were then restimulated with irradiated (50 Gy) autologous B-LCLs and CBZ for approximately 4 to 5 cycles. The T-cell clones were obtained by means of serial dilution.[22] CTL TCLs were sorted by using FACSAria (BD, Franklin Lakes, NJ).

T-cell proliferation and cytotoxic assays

We used the TCLs for pulsing experiments, cross-reactivity, HLA restriction, and antigen processing. Briefly, the TCLs (10^4 cells) were incubated with irradiated APCs (10^3 cells) and drugs for 48 hours, and the proliferation of TCLs was measured based on incorporation of tritiated thymidine (Perkin-Elmer, Waltham, Mass) for 16 hours. For the fixation assay, the autologous B-LCLs were pretreated with 0.25% paraformaldehyde for 30 minutes. Proliferative responses of T cells are shown as stimulation indices (in counts per minute in the drug-treated group/counts per minute in the vehicle-treated group). For the cytotoxicity assay, we first labeled APCs with ^{51}Cr (Perkin-Elmer) and then incubated the APCs with different drugs and TCLs or T-cell clones with an effector cells/target cells ratio of 5:1 to 40:1.[22] For the antibody blockage assay, autologous B-LCLs were pretreated with HLA class I antibody (W6/32; BioLegend, San Diego, Calif) or class II antibody (IVA12). For pulsing experiments, the irradiated autologous B-LCLs were first pulsed with the drugs overnight, and then the drugs were washed away before performing T-cell cytotoxicity assays.

Surface plasmon resonance measurement and analyses

Complexes of the soluble recombinant proteins HLA-B, endogenous peptide, and β_2-microglobulin were purified from the culture medium of C1R transfectants by using an affinity column coated with w6/32 antibody, as previously described.[23] We used the Biacore T100 surface plasmon resonance (SPR) biosensor (GE Healthcare, Piscataway, NJ) for analyzing the interaction between HLA-B proteins and drugs (see Fig E1). Briefly, we immobilized the purified soluble HLA-B proteins (acting as ligands) on the chips by means of an amine-coupling reaction, and the immobilized levels of sHLA-Bs were 9373-9812 response units. The drugs dissolved in PBS with 5% dimethyl sulfoxide were used for analysis and flowed through the solid phase. Responses of the interaction were reference subtracted and corrected with a standard curve for the dimethyl sulfoxide effects. We used BIA Evaluation Version 3.1 for data analysis.

HLA-B*1502 peptide–binding assay

We used 16 synthetic peptides (see Table E2 in this article's Online Repository at www.jacionline.org) to investigate the CBZ/peptide/HLA-B*1502 interaction based on CTL cytotoxic activity. A C1R-f1502 stable clone was generated by means of transfection of the full-length cDNA plasmid encoding HLA-B*1502.[23] The endogenous peptides in HLA-B*1502 were removed by using cold mild citric acid.[24] After neutralizing with cultured medium, the cells were incubated with β_2-microglobulin (4 μg/mL), GolgiStop (1 μL/mL; BD Biosciences, San Jose, Calif), and the synthetic peptides, respectively, for 3 hours at room temperature. The reconstitution was detected by using anti–HLA class I antibody (w6/32) with flow cytometry.

In silico modeling of drugs and the peptide-loaded HLA-B*1502 complex

A homology protein model of peptide-loaded HLA-B*1502 (accession no. BAA08824) was constructed by using HLA-B*1501 protein (PDB ID: 1XR8) as the template structure. The candidate chemical binding sites were predicted by using the DS::MCSS and DS::Flexible Docking protocol (Discovery Studio version 2.5). Methods describing detailed information of computer modeling can be found in the Methods section in this article's Online Repository at www.jacionline.org.